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TITLE: Compositions and methods for determining anti-viral drug susceptibility and
resistance and anti-viral drug screening
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INVENTOR-INFORMATION:

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CLAIMS:

What is claimed is:
1. A method for determining susceptibility for an anti-HIV drug comprising:
(a) introducing a resistance test vector comprising a patient-derived segment and an
indicator gene into a host cell;
(b) culturing the host cell from (a);
(c) measuring expression of the indicator gene in a target host cell, wherein the
expression of the indicator gene is dependent upon the patient-derived segment; and
(d) comparing the expression of the indicator gene from (c) with the expression of
the indicator gene measured when steps (a)-(c) are carried out in the absence of the
anti-HIV drug,
wherein a test concentration of the anti-HIV drug is present at steps (a)-(c); at
steps (b)-(c); or at step (c).
2. The method of claim 1, wherein the resistance test vector comprises DNA of a
genomic viral vector.
3. The method of claim 1, wherein the resistance test vector comprises DNA of a

subgenomic viral vector.
4. A method for determining anti-HIV drug resistance in a patient comprising:
(a) developing a standard curve of drug susceptibility for an anti-HIV drug;
(b) determining anti-HIV drug susceptibility in the patient according to the method
of claim 1; and
(c) comparing the anti-HIV drug susceptibility in step (b) with the standard curve
determined in step (a), wherein a decrease in anti-HIV susceptibility indicates
development of anti-HIV drug resistance in the patient.
5. The method of claim 1, wherein the resistance test vector comprises DNA of HIV.
6. The method of claim 5, wherein the resistance test vector comprises DNA encoding
vif, vpr, tat, rev, vpu, and nef.
7. The method of claim 1, wherein the patient-derived segment comprises a
functional viral sequence.
8. The method of claim 1, wherein the patient-derived segment encodes one protein
that is the target of an anti-HIV drug.
9. The method of claim 1, wherein the patient-derived segment encodes two or more
proteins that are the targets of one or more anti-HIV drugs.
10. A method for determining anti-HIV drug resistance in a patient comprising:
(a) determining anti-HIV drug susceptibility in the patient at a first time according to the method of claim 1, wherein the patient-derived segment is obtained
from the patient at about said time;
(b) determining anti-HIV drug susceptibility of the same patient at a later time;
and
(c) comparing the anti-HIV drug susceptibilities determined in step (a) and (b),
wherein a decrease in anti-HIV drug susceptibility at the later time compared to the
first time indicates development or progression of anti-HIV drug resistance

in the
patient.
11. The method of claim 1, wherein the patient-derived segment comprises an HIV
gene.
12. The method of claim 11, wherein the patient-derived segment comprises an HIV
gag-pol gene.
13. The method of claim 1, wherein the indicator gene is a functional indicator
gene and the host cell is a resistance test vector host cell, including the additional step of infecting the target host cell with resistance test vector viral
particles using filtered supernatants from said resistance test vector host cells.
14. The method of claim 1, wherein the indicator gene is a non-functional indicator
gene.
15. The method of claim 14, wherein the host cell is a packaging host cell/resistance test vector host cell.
16. The method of claim 15, wherein the culturing is by co-cultivation.
17. The method of claim 15, wherein the target host cell is infected with resistance test vector viral particles using filtered supernatants from said packaging host cell/resistance test vector host cells.
18. The method of claim 1, wherein the indicator gene is a luciferase gene.
19. The method of claim 1, wherein the indicator gene is an E. coli lacZ gene.
20. The method of claim 15, wherein the packaging host cell/resistance test vector
host cell is a human cell.
21. The method of claim 15, wherein the packaging host cell/resistance test vector
host cell is a human embryonic kidney cell.
22. The method of claim 15, wherein the packaging host cell/resistance test vector
host cell is a 293 cell.
23. The method of claim 1, wherein the target host cell is a human T cell.
24. The method of claim 1, wherein the target host cell is a human T cell leukemia

cell line.
25. The method of claim 1, wherein the target host cell is a Jurkat cell line.
26. The method of claim 1, wherein the target host cell is a H9 cell line.
27. The method of claim 1, wherein the target host cell is a CEM cell line.
28. The method of claim 1, wherein the target host cell is a human embryonic kidney
cell.
29. The method of claim 1, wherein the target host cell is a 293 cell.
30. A resistance test vector comprising an HIV patient-derived segment and an
indicator gene, wherein the expression of the indicator gene is dependent upon the
patient-derived segment.
31. The resistance test vector of claim 30, wherein the patient-derived segment
encodes one protein that is the target of an anti-HIV drug.
32. The resistance test vector of claim 30, wherein the patient-derived segment
encodes two or more proteins that are the targets of one or more anti-HIV drugs.
33. The resistance test vector of claim 30, wherein the patient-derived segment
comprises an HIV gene.
34. The resistance test vector of claim 30, wherein the patient-derived segment
comprises DNA of HIV.
35. The resistance test vector of claim 34, wherein the patient-derived segment
comprises DNA encoding vif, vpr tat, rev, vpu, and nef.
36. The resistance test vector of claim 33, wherein the patient-derived segment
comprises an HIV gag-pol gene.
37. The resistance test vector of claim 30, wherein the indicator gene is a functional indicator gene.
38. The resistance test vector of claim 30, wherein the indicator gene is a non-functional indicator gene.
39. The resistance test vector of claim 30, wherein the indicator gene is a luciferase gene.
40. A packaging host cell transfected with a resistance test vector of claim 30.

41. The packaging host cell of claim 40 that is a mammalian host cell.
42. The packaging host cell of claim 40 that is a human host cell.
43. The packaging host cell of claim 40 that is a human embryonic kidney cell.
44. The packaging host cell of claim 40 that is 293 cells.
45. The resistance test vector of claim 30 comprising an indicator gene viral vector and a packaging vector said indicator gene viral vector comprising an indicator gene and said packaging vector comprising a patient-derived segment.
46. A method for determining susceptibility for an anti-HIV drug comprising:
(a) introducing a resistance test vector comprising a patient-derived segment and a nonfunctional indicator gene into a host cell;
(b) culturing the host cell from (a);
(c) measuring expression of the indicator gene in a target host cell, wherein the expression of the indicator gene is dependent upon the patient-derived segment; and
(d) comparing the expression of the indicator gene from (c) with the expression of the indicator gene measured when steps (a)-(c) are carried out in the absence of the anti-HIV drug,
wherein a test concentration of the anti-HIV drug is present at steps (a)-(c); at steps (b)-(c); or at step (c).
47. The method of claim 46, wherein the resistance test vector comprises DNA of a genomic viral vector.
48. The method of claim 46, wherein the resistance test vector comprises DNA of a subgenomic viral vector.
49. The method of claim 46, wherein the resistance test vector comprises DNA of HIV.
50. The method of claim 49, wherein the resistance test vector comprises DNA

encoding vif, vpr, tat, rev, vpu, and nef.
51. The method of claim 46, wherein the patient-derived segment encodes one protein
that is the target of an anti-HIV drug.
52. The method of claim 46, wherein the patient-derived segment encodes two or more
proteins that are the targets of one or more anti-HIV drugs.
53. The method of claim 46, wherein the patient-derived segment comprises an HIV
gene.
54. The method of claim 53, wherein the patient-derived segment comprises an HIV
gag-pol gene.
55. The method of claim 46, wherein the indicator gene is a luciferase gene.
56. The method of claim 46, wherein the host cell is a packaging host cell.
57. The method of claim 46, wherein the packaging host cell is a human cell.
58. The method of claim 46, wherein the packaging host cell is a human embryonic
kidney cell.
59. The method of claim 46, wherein the packaging host cell is a 293 cell.
60. The method of claim 46, wherein the nonfunctional indicator gene comprises a
permuted promoter.
61. The method of claim 46, wherein the nonfunctional indicator gene comprises a
permuted coding region.
62. The method of claim 46, wherein the nonfunctional indicator gene comprises an
inverted intron.
63. The method of claim 46, wherein the host cell and target host cell are the same
cell.
64. The method of claim 46, wherein the target host cell is a human cell.
65. The method of claim 46, wherein the target host cell is a human T cell.
66. The method of claim 46, wherein the target host cell is a human embryonic
kidney cell.
67. The method of claim 46, wherein the target host cell is a 293 cell.

68. The method of claim 46, wherein the target host cell is infected with resistance test vector viral particles using filtered supernatants from said packaging host cell/resistance test vector host cell.
69. The method of claim 46, wherein said culturing is by co-cultivation.
70. A method for determining anti-HIV drug resistance in a patient comprising:
(a) developing a standard curve of drug susceptibility for an anti-HIV drug;
(b) determining anti-HIV drug susceptibility in the patient according to the method
of claim 46; and
(c) comparing the anti-HIV drug susceptibility in step (b) with the standard curve
determined in step (a), wherein a decrease in anti-HIV susceptibility indicates
development of anti-HIV drug resistance in the patient.
71. A method for determining anti-HIV drug resistance in a patient comprising:
(a) determining anti-HIV drug susceptibility in the patient at a first time according to the method of claim 46, wherein the patient-derived segment is obtained
from the patient at about said time;
(b) determining anti-HIV drug susceptibility of the same patient at a later time;
and
(c) comparing the anti-HIV drug susceptibilities determined in steps (a) and (b),
wherein a decrease in anti-HIV drug susceptibility at the later time compared to the
first time indicates development or progression of anti-HIV drug resistance in the
patient.
72. A method for evaluating the biological effectiveness of a candidate anti-HIV
drug compound comprising:
(a) introducing a resistance test vector comprising a patient-derived segment and an
indicator gene into a host cell;
(b) culturing the host cell from step (a);
(c) measuring expression of the indicator gene in a target host cell, wherein the

expression of the indicator gene is dependent upon the patient- derived segment;
and
(d) comparing the expression of the indicator gene from step (c) with the expression
of the indicator gene measured when steps (a)-(c) are carried out in the absence of
the candidate anti-HIV drug compound,
wherein a test concentration of the candidate anti-HIV drug compound is present at
steps (a)-(c); at steps (b)-(c); or at step (c).
73. The method of claim 72, wherein the resistance test vector comprises DNA of
HIV.
74. The method of claim 72, wherein the resistance test vector comprises DNA
encoding HIV gag-pol.
75. The method of claim 72, wherein the patient-derived segment encodes one protein
that is the target of an anti-HIV drug.
76. The method of claim 72, wherein the patient-derived segment encodes two or more
proteins that are the targets of one or more anti-HIV drugs.
77. The method of claim 72, wherein the patient-derived segment comprises an HIV
gene.
78. A method for determining susceptibility for an anti-HIV drug comprising:
(a) introducing a resistance test vector comprising a patient-derived segment and an
indicator into a host cell;
(b) culturing the host cell from (a);
(c) measuring the indicator in a target host cell, wherein a change in the indicator
is dependent upon the patient-derived segment; and
(d) comparing the measurement of the indicator from (c) with the measurement of the
indicator when steps (a)-(c) are carried out in the absence of the anti-HIV drug,
wherein a test concentration of the anti-HIV drug is present at steps (a)-(c);

at
steps (b)-(c); or at step (c).
79. The method of claim 78, wherein the indicator comprises a DNA structure.
80. The method of claim 78, wherein the indicator comprises a RNA structure.
81. A method for evaluating the biological effectiveness of a candidate anti-HIV
drug compound comprising:
(a) introducing a resistance test vector comprising a patient-derived segment and an
indicator into a host cell;
(b) culturing the host cell from step (a);
(c) measuring the indicator in a target host cell, wherein a change in the indicator
is dependent upon the patient-derived segment; and
(d) comparing the measurement of the indicator from step (c) with the measurement of
the indicator measured when steps (a)-(c) are carried out in the absence of the
candidate anti-HIV drug compound, wherein a test concentration of the candidate
anti-HIV drug compound is present at steps (a)-(c); at steps (b)-(c); or at step
(c).
82. The method of claim 81, wherein the indicator comprises a DNA structure.
83. The method of claim 82, wherein the indicator comprises a RNA structure .

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CLAIMS:

What is claimed is:
1. A method for determining susceptibility for an anti-HBV drug comprising:
(a) introducing a resistance test vector comprising a patient-derived segment and an
indicator gene into a host cell;
(b) culturing the host cell from (a);
(c) measuring expression of the indicator gene in a target host cell, wherein the
expression of the indicator gene is dependent upon the patient- derived seqment;
and
(d) comparing the expression of the indicator gene from (c) with the expression of
the indicator gene measured when steps (a)-(c) are carried out in the absence of the
anti-HBV drug, wherein a test concentration of the anti-HBV drug is present at steps
(a)-(c); at steps (b)-(c); or at step (c).
2. The method of claim 1 wherein the indicator gene is a non-functional indicator
gene.
3. The method of claim 1 wherein the indicator gene is a luciferase gene.

4. The method of claim 1 wherein the indicator gene is an E. coli lacz gene.
5. The method of claim 1 wherein the target host cell is a Jurkat cell line.
6. A resistance test vector comprising a HBV patient-derived segment and an
indicator gene.
7. The resistance test vector of claim 6 wherein the indicator gene is a functional
indicator gene.
8. The resistance test vector of claim 6 wherein the indicator gene is a non-functional indicator gene.
9. The resistance test vector of claim 6 wherein the indicator gene is a luciferase
gene.
10. A packaging host cell transfected with a resistance test vector of claim 6.
11. The packaging host cell of claim 10 that is a mammalian host cell.
12. The packaging host cell of claim 10 that is a human host cell.
13. The packaging host cell of claim 10 that is a human embryonic kidney cell.
14. The packaging host cell of claim 10 that is 293 cells.
15. The packaging host cell of claim 10 that is a human hepatoma cell line.
16. The packaging host cell of claim 10 that is HepG2.
17. The packaging host cell of claim 10 that is Huh7.
18. A method for determining susceptibility for an anti-HBV drug comprising:
(b) introducing a resistance test vector comprising a patient-derived segment and a
nonfunctional indicator gene into a host cell;
(b) culturing the host cell from (a);
(c) measuring expression of the indicator gene in a target host cell, wherein the
expression of the indicator gene is dependent upon the patient- derived segment;
and
(d) comparing the expression of the indicator gene from (c) with the expression of
the indicator gene measured when steps (a)-(c) are carried out in the absence of the

anti-HBV drug, wherein a test concentration of the anti-HBV drug is present at steps
(a)-(c); at steps (b)-(c); or at step (c).
19. The method of claim 18 wherein the nonfunctional indicator gene comprises a
permuted promoter.
20. The method of claim 18 wherein the nonfunctional indicator gene comprises a
permuted coding region.
21. The method of claim 18 wherein the nonfunctional indicator gene comprises an
inverted intron.
22. The method of claim 18 wherein the host cell and target cell are the same cell.
23. The method of claim 18 wherein the target cell is a human cell.
24. A method for determining anti-HBV drug resistance in a patient comprising:
(a) developing a standard curve of drug susceptibility for an anti-HBV drug;
(b) determining anti-HBV drug susceptibility in the patient according to the method
of claim 1; and
(c) comparing the anti-HBV drug susceptibility in step (b) with the standard curve
determined in step (a), wherein a decrease in anti-HBV susceptibility indicates
development of anti-HBV drug resistance in the patient.
25. A method for determining anti-HBV drug resistance in a patient comprising:
(a) developing a standard curve of drug susceptibility for an anti-HBV drug;
(b) determining anti-HBV drug susceptibility in the patient according to the method
of claim 18; and
(c) comparing the anti-HBV drug susceptibility in step (b) with the standard curve
determined in step (a), wherein a decrease in anti-HBV susceptibility indicates
development of anti-HBV drug resistance in the patient.
26. A method for determining anti-HBV drug resistance in a patient comprising:
(a) determining anti-HBV drug susceptibility in the patient at a first time

according to the method of claim 1, wherein the patient-derived segment is obtained
from the patient at about said time;
(b) determining anti-HBV drug susceptibility of the same patient at a later time;
and
(c) comparing the anti-HBV drug susceptibilities determined in step (a) and (b),
wherein a decrease in anti-HBV drug susceptibility at the later time compared to the
first time indicates development or progression of anti-viral drug resistance in the
patient.
27. A method for determining anti-HBV drug resistance in a patient comprising:
(a) determining anti-HBV drug susceptibility in the patient at a first time according to the method of claim 18, wherein the patient-derived segment is obtained
from the patient at about said time;
(b) determining anti-HBV drug susceptibility of the same patient at a later time;
and
(c) comparing the anti-HBV drug susceptibilities determined in steps (a) and (b),
wherein a decrease in anti-HBV drug susceptibility at the later time compared to the
first time indicates development or progression of anti-HBV drug resistance in the
patient.
28. The method of claim 1 wherein the resistance test vector comprises DNA encoding
C, P, and X.
29. The method of claim 1 wherein the patient-derived segment comprises a P gene.
30. The method of claim 1 wherein the patient-derived segment comprises an HBV
gene.
31. The method of claim 1 wherein the patient-derived segment comprises an HBV RT
gene.

32. The method of claim 1 wherein the patient-derived segment comprises an HBV DNA
polymerase gene.
33. The resistance test vector of claim 6 wherein the patient-derived segment
comprises an HBV P gene.
34. A method for evaluating the biological effectiveness of a candidate anti-HBV
drug compound comprising:
(a) introducing a resistance test vector comprising a patient-derived segment and an
indicator gene into a host cell;
(b) culturing the host cell from step (a);
(c) measuring expression of the indicator gene in a target host cell, wherein the
expression of the indicator gene is dependent upon the patient-derived segment; and
(d) comparing the expression of the indicator gene from step (c) with the expression
of the indicator gene measured when steps (a)-(c) are carried out in the absence of
the candidate anti-HBV drug compound, wherein a test concentration of the candidate
anti-HBV drug compound is present at steps (a)-(c) at steps (b)-(c); or at step
(c).
35. The method of claim 34 wherein the resistance test vector comprises DNA
encoding HBV P protein.
36. The method of claim 34 wherein the patient-derived segment comprises an HBV
gene.
37. A method for determining susceptibility for an anti-HBV drug comprising:
(a) introducing a resistance test vector comprising a patient-derived segment and an
indicator into a host cell;
(b) culturing the host cell from (a);
(c) measuring the indicator in a target host cell, wherein a change in the indicator

is dependent upon the patient-derived seqment; and
(d) comparing the measurement of the indicator from (c) with the measurement of the
indicator when steps (a)-(c) are carried out in the absence of the anti-HBV drug,
wherein a test concentration of the anti-viral drug is present at steps (a)-(c); at
steps (b)-(c); or at step (c).
38. The method of claim 37 wherein the indicator comprises a DNA structure.
39. The method of claim 37 wherein the indicator comprises a RNA structure.
40. A method for evaluating the biological effectiveness of a candidate anti-HBV
drug compound comprising:
(a) introducing a resistance test vector comprising a patient-derived segment and an
indicator into a
(b) culturing the host cell from step (a);
(c) measuring the indicator in a target host cell, wherein a change in the indicator
is dependent upon the patient-derived segment; and comparing the measurement of the
indicator from step(c) with the measurement of the indicator measured when steps
(a)-(c) are carried out in the absence of the candidate anti-HBV drug compound,
wherein a test concentration of the candidate anti-viral drug compound is present at
steps (a)-(c); at steps(b)-(c); or at step (c).
41. The method of claim 40 wherein the indicator comprises a DNA structure.
42. The method of claim 40 wherein the indicator comprises a RNA structure.